Hindawi Publishing Corporation
International Journal of Ecology
Volume 2015, Article ID 390591, 7 pages
http://dx.doi.org/10.1155/2015/390591

Review Article

^{13}C NMR and ESR Characterization of Humic Substances Isolated from Soils of Two Siberian Arctic Islands

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Received 23 July 2015; Revised 19 October 2015; Accepted 25 October 2015

Academic Editor: Ram Chander Sihag

Humic acids (HAs) and fulvic acids (FAs) of two Polar soils were investigated by ^{13}C NMR and ESR spectroscopies, investigating the degree of humification and the molecular structure. One soil, from Bolshoi Lyakhovsky Island, contains two humus horizons: modern and buried. The other soil, from Wrangel Island, had only one modern humus horizon. The HAs and FAs of the two soils investigated show essential differences. The HAs show fewer oxygen-containing groups in comparison with the FAs, whereas the degree of aromaticity is two or three times higher in the HAs. The ^{13}C NMR data also show that HAs are very different from FAs in terms of their molecular composition and hydrophobicity. Humification in the Arctic is limited by the very low content of lignin-derived compounds, due to the restricted vascular flora. As a result, the HAs, isolated from Polar soils, are more similar to the corresponding FAs than to the typical HAs of temperate soils. This was confirmed by ESR data, which show similar levels of free radical concentration for HAs and FAs and are related to the low level of aromaticity of both materials investigated. Apparently, the humification process in the soils of Polar Arctic deserts is in an initial stage.

1. Introduction

Soils sublayered by permafrost are widely distributed in cold environments and prevail in the northern hemisphere. Permafrost affects the soil genesis, morphology and also regulates the main chemical and physical processes that determine the structure of the soil cover. Permafrost soils are known to have low degree of humification [1] in comparison with temperate ones, but the question of biodegradability of organic matter in these soils under conditions of climate change has not yet been answered. Soils affected by permafrost are typical for Canada, Greenland, Scandinavia, Russia, China, and Mongolia [2]. Not all of them are Polar soils, because the permafrost of Holocene/Pleistocene age is situated at latitudes more southern than 60 N, mainly in Eastern Siberia, and partially in Mongolia and China. The diversity of permafrost-affected soils is based on the cryopedogenesis process. Polar soils play a key role in the global carbon balance and provide maximum stocks of soil organic matter in the whole pedosphere. Humification is a global process which occurs even in the very severe climatic conditions of the Arctic and Antarctic [3]. There are two limiting factors for humification in permafrost-affected soils: the period of soil biological activity and the presence of lignin-derived compounds as precursors of humification [1]. The period where the temperature is above 0°C varies from a few days to a few months in different zones of both Polar regions. Organic horizons contain an essential part of phenylpropanoic and lignin-derived compounds in the case of Arctic soils, but these values are very low in Antarctic ones. Another difference is in the stocks of total soil organic matter (SOM), which is higher in polar soils of the northern hemisphere.

It is well known that the humification rate is affected by SOM stocks. Soils of the Yamal and Gydan peninsulas, as well as some soil of Eastern Siberia, have been investigated previously in terms of SOM quality and stocks [4–6]. Further Siberian soils were studied by ^{13}C NMR spectroscopy [7]. Nevertheless, humic substances isolated from soils of oceanic

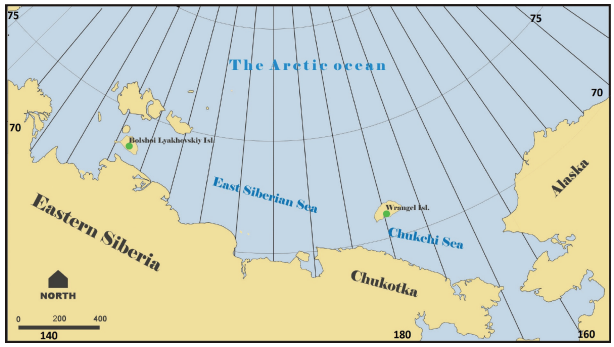

Figure 1: B. Lyakhovsky and Wrangel Islands on the map of Northern Siberia.

islands of the Russian Arctic have not been studied previously for their structural composition. This work aims to investigate the humic substances of Polar desert soils region by ^{13}C NMR and ESR spectroscopies. The first method allows us to assess the molecular structure of humic substances, while the second one provides data about their paramagnetic activity.

2. Materials and Methods

2.1. Study Sites and Soil Samples. Soil samples were collected in the Eastern Arctic part of the Bolshoi Lyakhovsky and Wrangel Islands (Figure 1). Soils were sampled from A horizons in both cases and from a buried [H] layer in B. Lyakhovsky Island.

Bolshoi Lyakhovsky Island (73°21,660′ N, 139°56,132′ E) is a part of the Novosibirsk Islands archipelago in the Laptev Sea. The maximum elevation of the island is about 300 m. The relief is flat and characterized by plains containing friable clay and sandy-clay deposits containing thick layers of fossil ice and abundant bone remains of mammoth fauna. Bolshoi Lyakhovsky Island belongs to the Siberian climate region of the Russian Arctic. The snow cover is stable here during nine months each year. The average temperature in January is between −28°C and −31°C; in July it is about +3°C. The annual rainfall is less than 77 mm, whereas the total annual precipitation is about 140 mm. Patterned grounds form the microrelief in B. Lyakhovsky. Vegetation cover is dense and dominated by Gramíneae (*Alopecurus alpinus, Poa alpigena*) and other arctic-alpine flora such as *Cerastium sp*.

Wrangel Island (70°59,172′ N, 178°28,037′ W) is one of the largest islands in the region of the Chukotskoye Sea, with an area of about 7600 sq km. The main part of the mountain relief is characterized by flat-topped mountains 400–600 m in height. Mountains are surrounded by flat, gently sloping plains covered by lake-alluvial deposits. Wrangel Island is located on the border of the Siberian and Eastern (Pacific) climatic regions of the Russian Arctic. The average annual temperature on Wrangel Island is −11.5°C. The minimum temperature is −43°C; the maximum is +18.2°C. The annual precipitation is about 92 mm, with a maximum of 150 mm, and winter is about 7-8 months long. Vegetation in the studied area is irregular, not very dense and presents mainly lichens and some vascular plants, with some examples of *Salix* sp. present.

The soils investigated are classified as Cryosols or Gelisols (Figure 2). Many of the permafrost-affected soils are considered to be Crysols or Cryozems. The Russian soil classification identifies Cryozems [8] as soils affected by permafrost and presenting morphological features of cryoturbation. Other soils that do not present cryoturbation morphological features are classified as soils of other classes or orders with an additional verificator "Cryozem." The "keys to soil taxonomy" [9] have a class of Gelisols and the WRD system contains the Cryosol group [10]. The term "Gelisols" is broader than Cryosol, while the term Cryozems is best applied in terms of morphology. In any case, Cryosols and Cryozems, as parts of Gelisol units, are typical soil covers of Siberia and these soils have shown intense changes in morphology and thermic regime in the last decades.

2.2. Humic Substances Isolation. Soil carbon content was not less than 1% in all the samples investigated. The HAs and FAs were extracted according to the procedure recommended by the IHSS [11]. Briefly, HAs were extracted with 0.1 M NaOH (soil/solution ratio 1 : 10) under nitrogen gas. After 24 h of shaking, the alkaline supernatant was separated from the soil residue by centrifugation at 1,516 ×g for 20 min and acidified to pH 1 with 6 M HCl to induce the precipitation of HAs. The supernatant, which contained FA, was separated from the precipitate (HAs) by centrifugation at 1,516 ×g for 15 min. The HAs were then redissolved in 0.1 M NaOH and shaken for 4 h under N_2 before the suspended solids were removed by centrifugation. The HA solution was acidified again with 6 M HCl to pH 1.0 and the HAs were separated by centrifugation. The HAs were demineralized by shaking overnight in 0.1 M HCl/0.3 M HF (solid/solution ratio 1 : 1) and then repeatedly washed with deionized water until the washing reached pH 3. The solid HAs were then freeze-dried. The FA preparations were purified on activated carbon AG-3 according to Forsyth and desalinated on cationic KU-2 (H^+ form).

2.3. ^{13}C NMR Spectroscopy. The ^{13}C NMR spectra of the air-dried HA and FA samples were recorded on a JNM-ECA 400 NMR spectrometer (JEOL, Japan) with a working frequency of 100.53 MHz using the CP-MAS procedure. The spinning speed was 6 kHz; the contact time was 5 ms and the recycle delay was 5 s. The chemical shifts have been referenced to tetramethylsilane at 0 ppm. The quantitative processing was performed by numerical integration in the regions corresponding to the functional groups and molecular fragments with preliminary automatic correction of the phase and the baseline using Delta v. 5.0.2. (JEOL, Japan).

Groups of carbon species have been identified by the intervals of chemical shift (Table 1). Standardization of quantitative characteristics of HAs and FAs acids was performed by the following parameters: the percentage of aromaticity – Ar/(Ar + AL) [12, 13] and the degree of organic matter decomposition (C,H-alkyl/O,N-alkyl) [13]. Moreover, an integral index of molecule hydrophobicity was used for humic substances ($AL_{H,R} + Ar_{H,R}$), which calculated the sum of unoxidized atoms of carbon [14]. This index provides information about the amphiphilic properties of humic substances. Signals of aromatic structures (Ar) were calculated

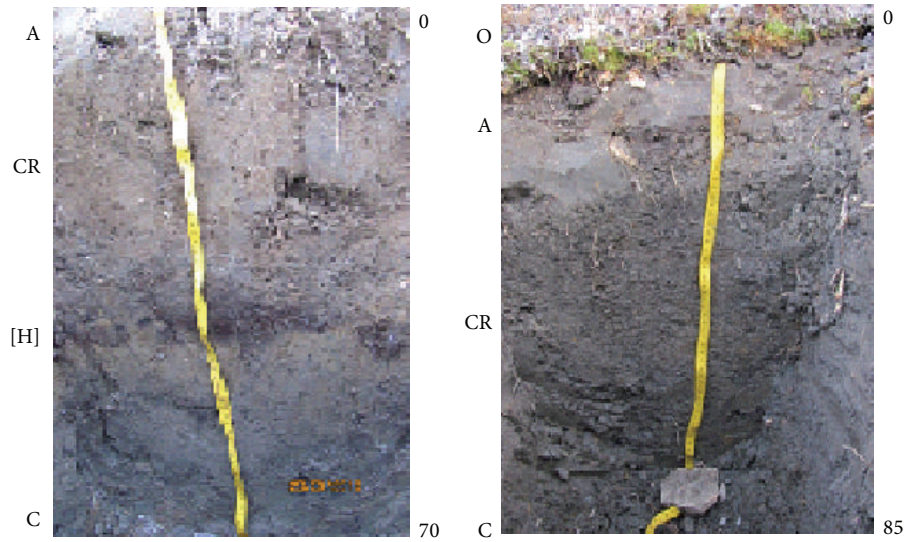

Figure 2: Soil profiles of the B. Lyakhovsky (a) and Wrangel (b) Islands.

Table 1: Carbon species and chemical shifts via ^{13}C NMR.

Chemical shift, ppm	Molecular fragments: carbon species
0–47	Unsubstituted saturated alkyl carbons (C)
47–60	Alkyl C substituted by oxygen and nitrogen atoms
60–110	Alkyl C singly bonded to one oxygen atom (such as ring C in carbohydrates) and alkyl C bonded to two oxygen atoms (such as anomeric C of carbohydrates)
110–144	Proton- and alkyl-substituted aromatic C
144–164	Oxygen-substituted aromatic C
164–183	Carboxyl, ester, and amide C
183–190	Quinone C

as a sum of carbon species in the ranges 110–164 and 183–190 ppm; the aliphatic (AL) sum was in the ranges 0–110 and 164–183 ppm intervals. $Ar_{H,R}$ were summed in the intervals of 0–47 and 110–144 ppm, C,H-alkylic signals corresponded to the 0–47 ppm shifts, and O,N-alkyls referred to the ranges of 47–60 and 60–110 ppm.

2.4. Electronic Paramagnetic Resonance Experiment. The ESR spectra were recorded on a JES FA 300 spectrometer (JEOL, Japan) in X-diapason with a free radical modulation amplitude of 0.06 mT and a microwave power in the cavity of 1 mW. Magnesium powder with fixed radical concentration was used as an external standard. The concentration of paramagnetic centers in powders was determined by comparison of relative signal intensities of the external standard and measured sample with the use of the program JES-FA swESR v. 3.0.0.1 (JEOL, Japan).

3. Results and Discussion

HAs isolated from Arctic soils are quite different from the FAs of the same layers on elemental as well as structural composition (Table 2). The HAs contain more carbon and nitrogen, whereas the FAs contain more oxygen. In the aliphatic carbon region, all the HAs spectra exhibit one relatively sharp peak centred at 30 ppm, which can be tentatively assigned to methylene C at positions α, β, δ, and ε from terminal methyl groups (15 ppm) in alkyl chains [15] (Figures 3 and 4). These methylene C atoms are believed to arise from the recalcitrance and accumulation of waxes, lipids, cutin, and suberin polymers from plants [16–18]. All spectra also exhibit a peak at 55 ppm, which is due to methoxyl groups and it is normally associated with lignin-derived structures such as those of syringyl and guaiacyl units [19]. Nevertheless, resonance in this chemical shift region may also derive from C at position α in polypeptides $(-C(O)-C^{*}(R)H-NH-)_n$ [17, 20].

A very intense signal at 71 ppm in the spectra is characteristic of carbons in CH(OH) groups, such as those of the ring carbons of carbohydrates [15]. Resonance in this spectral region spreads from 64 to 90 ppm, which is likely to originate from the various HC–OH fragments of cellulose or other carbohydrate structures [17]. Besides this spectral signature, additional signals of carbohydrate-like structures were further identified in the majority of the HA spectra. The weak resonance at approximately 62 ppm is typical of CH_2O-groups (C6 carbons) of hexoses units from polysaccharides [21]. The presence of a downfield resonance at 101 ppm, which is representative of anomeric C, further corroborates the presence of carbohydrate-like moieties in the soil HA and FA samples. The most intensive signals are related to the carbohydrates area of the FAs.

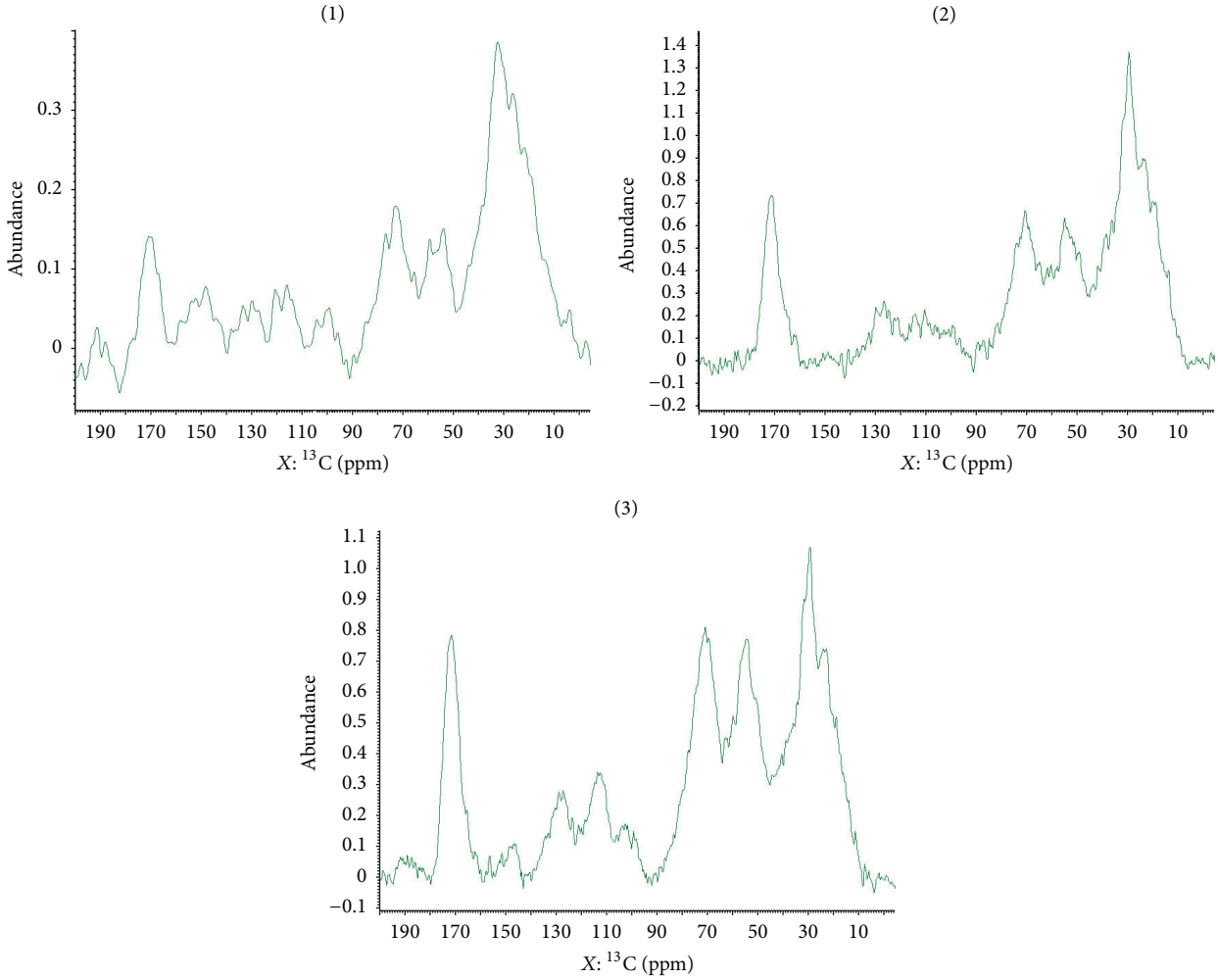

Figure 3: ^{13}C NMR spectra of HAs isolated from Arctic soils: buried H layer (1), uppermost A horizon Bolshoy Lyakhovsky Island (2) and A horizon, Wrangel Island (3).

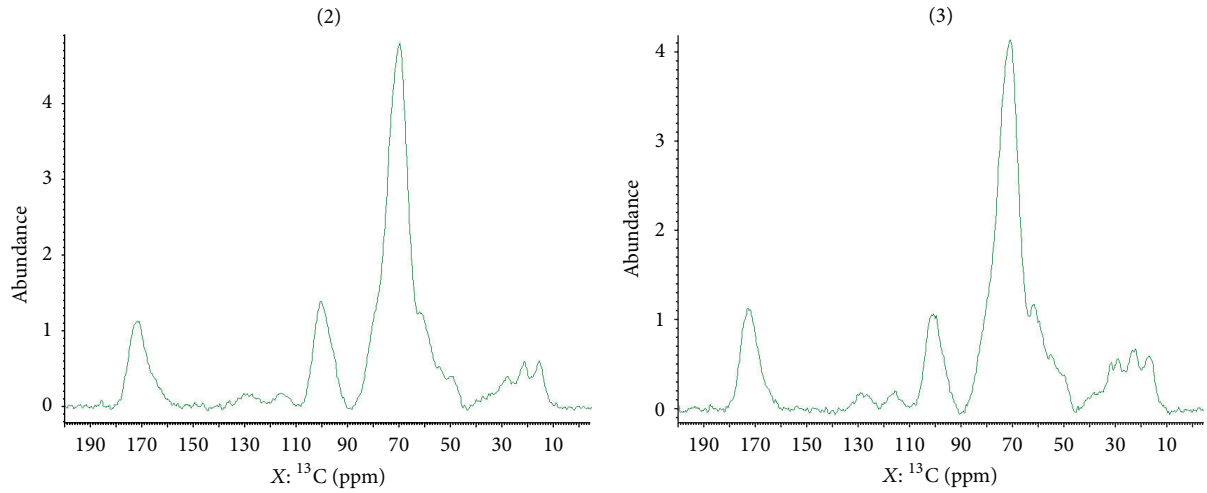

Figure 4: ^{13}C NMR spectra of FAs isolated from Arctic soils; numbers are the same as those in Figure 1.

Table 2: Elemental composition of HAs and FAs (all results are given for ash-free preparations).

Plot, horizons, and depths	Ash, %	Weight portion, %			
		C	H	O	N
		Humic acids			
Bolshoy Lyakhovsky, A 0–3 cm	3,20 ± 0,16	49,9 ± 1,6	5,6 ± 0,5	39,0 ± 5,0	5,6 ± 0,4
Bolshoy Lyakhovsky [H], buried	2,16 ± 0,11	49,6 ± 1,6	4,8 ± 0,4	42,0 ± 5,1	3,7 ± 0,3
Wrangel Island, A, 0–5 cm	2,75 ± 0,14	48,1 ± 1,5	5,0 ± 0,5	42,0, ± 3,2	4,9 ± 0,3
		Fulvic acids			
Bolshoy Lyakhovsky, A 0–3 cm	1,44 ± 0,07	40,4 ± 1,3	5,1 ± 0,5	52,1 ± 1,9	2,4 ± 0,4
Wrangel Island, A, 0–5 cm	2,06 ± 0,10	42,2 ± 1,4	5,2 ± 0,5	49,7 ± 6,4	3,0 ± 0,2

Table 3: Percentage of carbon in the main structural fragments of HAs and FAs (according to ^{13}C NMR).

Plot, horizons, and depths	Chemical shifts, ppm						
	0–47	47–60	60–110	110–144	144–164	164–183	183–190
			Humic acids				
Bolshoy Lyakhovsky, A 0–3 cm	44,5	12,0	21,2	10,0	1,2	10,9	0,2
Bolshoy Lyakhovsky [H], buried	49,7	9,4	17,5	10,3	5,8	7,1	0,2
Wrangel Island, A, 0–5 cm	33,8	16,5	24,8	11,8	1,4	11,2	0,5
			Fulvic acids				
Bolshoy Lyakhovsky, A 0–3 cm	9,7	6,3	69,3	3,6	1,1	9,9	0,1
Wrangel Island, A, 0–5 cm	12,9	7,2	65,3	3,6	0,5	10,4	0,1

In the aromatic region, the resonance within the chemical shift region between 105 and 144 ppm may originate from unsubstituted and alkyl-substituted aromatic C. The signal at approximately 149 ppm is typical in spectra of lignin structural units and it is attributed to oxygen-substituted aromatic ring C, such as those of syringyl and guaiacyl units [17, 19]. The NMR spectra of HAs show a substantial proportion of aromatic compounds, whereas this value is very low in FAs (Table 2).

The carboxyl region (164–183 ppm) is dominated by a peak with a maximum at approximately 171 ppm, largely attributed to carboxyl C but also to carbonyl C from amides and polypeptides [17, 22]. The comparative analyses of molecular structure and elemental composition show that FAs are enriched in oxygen components, which determine increased hydrophilicity and ability to migrate in aqueous solutions (Table 3). Similar results were obtained previously for soils of Alaska [23] and the Ural Mountains [24]. The high relative content of carboxylic groups in FAs would increase their migration ability and biogeochemical activity in Polar terrestrial ecosystems. Quinone C contributes to the very weak resonance at approximately 185 ppm.

The aromaticity index is two or three times higher in HAs than in FAs. In general, HAs are more stable to oxidation and are more condensed and aromatisized than FAs. The index of hydrophobicity ($AL_{H,R}$ + $Ar_{H,R}$) is higher in HAs than in FAs. In general, the humification degree, assessed by the C,H-alkyl/O,N-alkyl ratio is higher for HAs. Comparing HAs from the two soils, the HA from Bolshoi Lyakhovsky shows a higher degree of humification than that from Wrangel Island (Table 4).

The aromaticity degree and the hydrophobic level are higher in HAs than in FAs. These results support the evidence that the humification is a well expressed and intensive process in Polar deserts of the Russian Arctic, which results in the formation of two different groups of humic substances, HAs and FAs, which are differentiated in chemical composition and molecular structure. The FAs of the two soils investigated are more similar than the HAs in chemical composition.

Apparently, HAs from Arctic soils are less enriched in aromatic fragments than HAs from taiga soils [22]. The HAs investigated are different from the FAs and the main difference is in the oxygen content and the proportion of aliphatic fragments.

A typical ESR spectrum of HAs and FAs investigated is presented in Figure 5, and the ESR parameters are similar to HAs and FAs of temperate soils [25]. The spectra show a single wide line with a g factor ranging from 2.0032 to 2.0037, which is attributed to the presence of stable semiquinone free radicals in the HA and FA macromolecules (Table 5).

On average, the g-factor is higher in FAs than in HAs, which suggests that the free electron in FAs is more shifted towards the oxygen atom than in HAs.

4. Conclusions

Humic and fulvic acids of some Polar soils were investigated by ^{13}C NMR an ESR spectroscopies to reveal the humification degree and their specific molecular structure in Arctic soils. Humic substances of two soils investigated show a substantial difference in aromatic compound portion due to different levels of polymerization of HAs and FAs. HAs show the

Table 4: Integral indexes of molecular composition of HAs and FAs.

Plot, horizons, and depths	Aromaticity, %	$AL_{H,R}$ + $Ar_{H,R}$, %	C,H-alkyl/O,N-alkyl
	Humic acids		
Bolshoy Lyakhovsky, A 0–3 cm	11,4	54,5	1,3
Bolshoy Lyakhovsky, [H], buried	16,3	60,0	1,8
Wrangel Island, A, 0–5 cm	13,7	45,6	0,8
	Fulvic acids		
Bolshoy Lyakhovsky, A 0–3 cm	4,8	13,3	0,1
Wrangel Island, A, 0–5 cm	4,2	16,5	0,2

Table 5: Paramagnetic features of HAs and FAs.

Plot	Horizon, and depth, cm	Humic acids		Fulvic acids	
		Mass concentration of free radical, 10^{15} spin$*$g^{-1}	g-factor	Mass concentration of free radical, 10^{15} spin$*$g^{-1}	g-factor
Bolshoy Lyakhovsky	A 0–3	2,25	2,0032	2,13	2,0037
Bolshoy Lyakhovsky	[H]	0,85	2,0034	0,86	2,0032
Wrangel Island	A 0–5	1,78	2,0033	3,87	2,0034

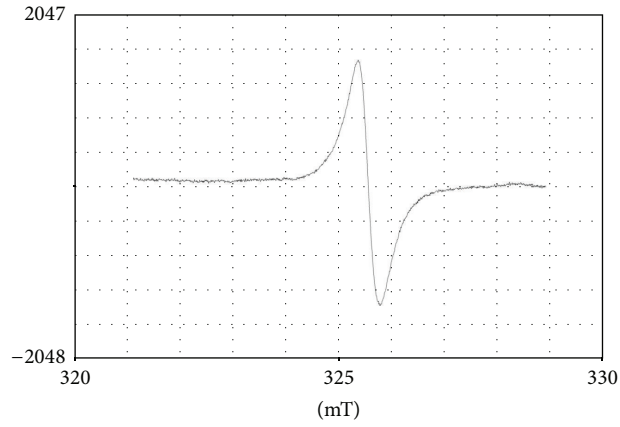

Figure 5: Typical ESR spectrum of humic substances investigated.

lower content of oxygen-containing groups in comparison with FAs, while the degree of aromaticity is two or three times higher than in FAs. The degree of aromaticity of buried organic material from Bolshoi Lyakhovsky Island is greater than that in the humus horizon of the modern soil.

The ^{13}C NMR data show that HAs are very different from FAs in terms of molecular composition, degree of aromaticity, and hydrophobicity. Humification in the Arctic appears to be very limited by the low content of compounds derived from the restricted vascular flora. As a result, the HAs from Polar soils are more similar to their corresponding FAs than they are to HAs from temperate soils. ESR data show that the levels of free radical concentration are comparable in HAs and FAs. The low free radical concentrations can be related to the low level of aromaticity of both HAs and FAs. Thus, the humification process in Polar Arctic desert soils can be considered to be in the initial stage, though leading to the formation of two differentiated groups of humic substances in soils, HAs and FAs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The work was funded by the Russian President Grant for Young Doctors of Science no. MD-3615.2015.4 and Russian Foundation for Basic Research, projects nos. 13-04-01693-a, 13-04-00070-a. This research was supported by the joint Russian-German project CARBOPERM. Scientific research was performed at the Center for Magnetic Resonance of Research Park of St. Petersburg State University.

References

[1] E. V. Abakumov, "The sources and composition of humus in some soils of West Antarctica," *Eurasian Soil Science*, vol. 43, no. 5, pp. 499–508, 2010.

[2] S. Zubrzycki, L. Kutzbach, and E.-M. Pfeiffer, "Permafrost-affected soils and their carbon pools with a focus on the Russian Arctic," *Solid Earth*, vol. 5, no. 2, pp. 595–609, 2014.

[3] G. Aiken, D. Mcknight, R. Harnish, and R. Wershaw, "Geochemistry of aquatic humic substances in the Lake Fryxell basin, Antarctica," *Biogeochemistry*, vol. 34, no. 3, pp. 157–188, 1996.

[4] V. D. Vasil'evskaja, *Pochvoobrazovanie v tundrah Srednej Sibiri*, Nauka, Moscow, Russia, 1980.

[5] V. M. Tomashunas and E. V. Abakumov, "The content of heavy metals in soils of Yamal peninsula and Belyi Island," *Gigiena I Sanitariya*, vol. 6, pp. 26–31, 2014.

[6] D. Y. Vlasov, E. V. Abakumov, V. M. Tomashunas, V. A. Krylenkov, and M. S. Zelenskaya, "Mycobiota of soils and antropogenic substrates of the Yamal peninsula," *Gigiena i Sanitariya*, vol. 5, pp. 49–51, 2014.

[7] M. P. Sartakov, "13-C NMR spectroscopy of soils of the middle Priobye," *Chemistry of Plant Raw Materials*, vol. 3, pp. 135–139, 2008.

[8] L. L. Shishov, V. D. Tonkonogov, I. I. Lebedeva, and M. I. Gerasimova, *Classification and Diagnosis of Russian Soils*, Dokuchaev Soil Institute, Moscow, Russia, 2004.

[9] Soil Survey Staff, *Keys to Soil Taxonomy*, United States Department of Agriculture & Natural Resources Conservation Service, Washington, DC, USA, 2010.

[10] FAO, "World reference base of soil resources," World Soil Resources Report 84, FAO, Rome, Italy, 1998.

[11] R. S. Swift, *Methods of Soil Analysis*, vol. 3, Soil Science Society of America, Madison, Wis, USA, 1996.

[12] B. C. Liang, E. G. Gregorich, M. Schnitzer, and H.-R. Schulten, "Characterization of water extracts of two manures and their absorption on soils," *Soil Science Society of America Journal*, vol. 60, no. 6, pp. 1758–1763, 1996.

[13] J. A. Pedersen, M. A. Simpson, J. G. Bockheim, and K. Kumar, "Characterization of soil organic carbon in drained thaw-lake basins of Arctic Alaska using NMR and FTIR photoacoustic spectroscopy," *Organic Geochemistry*, vol. 42, no. 8, pp. 947–954, 2011.

[14] E. D. Lodygin, V. A. Beznosikov, and R. S. Vasilevich, "Molecular composition of humic substances in tundra soils (^{13}C-NMR spectroscopic study)," *Eurasian Soil Science*, vol. 47, no. 5, pp. 400–406, 2014.

[15] A. J. Simpson and M. J. Simpson, "Nuclear magnetic resonance analysis of natural organic matter," in *Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*, N. Senesi, B. Xing, and P. M. Huang, Eds., pp. 589–650, John Wiley & Sons, Hoboken, NJ, USA, 2009.

[16] A. Winkler, L. Haumaier, and W. Zech, "Insoluble alkyl carbon components in soils derive mainly from cutin and suberin," *Organic Geochemistry*, vol. 36, no. 4, pp. 519–529, 2005.

[17] C. Keeler, E. F. Kelly, and G. E. Maciel, "Chemical-structural information from solid-state ^{13}C NMR studies of a suite of humic materials from a lower montane forest soil, Colorado, USA," *Geoderma*, vol. 130, no. 1-2, pp. 124–140, 2006.

[18] E. Alarcón-Gutiérrez, C. Floch, C. Augur, J. L. Petit, F. Ziarelli, and S. Criquet, "Spatial variations of chemical composition, microbial functional diversity, and enzyme activities in a Mediterranean litter (*Quercus ilex* L.) profile," *Pedobiologia*, vol. 52, no. 6, pp. 387–399, 2009.

[19] G. R. Hatfield, G. E. Maciel, O. Erbatur, and G. Erbatur, "Qualitative and quantitative analysis of solid lignin samples by carbon-13 nuclear magnetic resonance spectrometry," *Analytical Chemistry*, vol. 59, no. 1, pp. 172–179, 1987.

[20] M.-F. Dignac, H. Knicker, and I. Kögel-Knabner, "Effect of N content and soil texture on the decomposition of organic matter in forest soils as revealed by solid-state CPMAS NMR spectroscopy," *Organic Geochemistry*, vol. 33, no. 12, pp. 1715–1726, 2002.

[21] R. M. B. O. Duarte, A. M. S. Silva, and A. C. Duarte, "Two-dimensional NMR studies of water-soluble organic matter in atmospheric aerosols," *Environmental Science and Technology*, vol. 42, no. 22, pp. 8224–8230, 2008.

[22] E. D. Lodygin and V. A. Beznosikov, "The ^{13}C NMR study of the molecular structure of humus acids from podzolic and bog-podzolic soils," *Eurasian Soil Science*, vol. 36, no. 9, pp. 967–975, 2003.

[23] X. Y. Dai, C. L. Ping, R. Candler, L. Haumaier, and W. Zech, "Characterization of soil organic matter fractions of tundra soils in Arctic Alaska by carbon-13 nuclear magnetic resonance spectroscopy," *Soil Science Society of America Journal*, vol. 65, no. 1, pp. 87–93, 2001.

[24] A. Dymov, E. V. Zhangurov, and F. Hagedorn, "Soil organic matter composition along altitudinal gradients in permafrost affected soils of the Subpolar Ural Mountains," *Catena*, vol. 131, pp. 140–148, 2015.

[25] N. Senesi, V. D'Orazio, and G. Ricca, "Humic acids in the first generation of eurosoils," *Geoderma*, vol. 116, no. 3-4, pp. 325–344, 2003.